FIGURE 1 The Hairpin Ribozyme

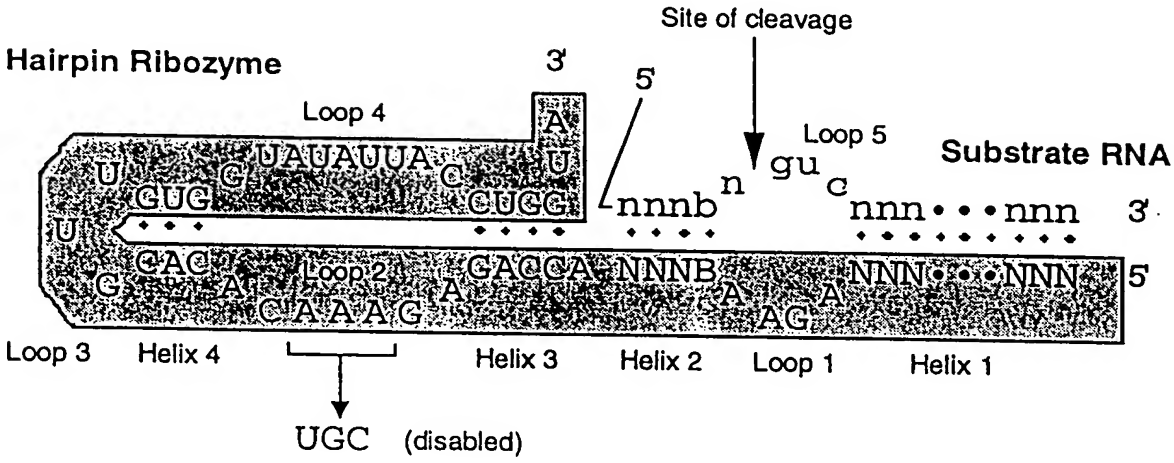


FIGURE 2

Cleavage of target substrates by hairpin Rz library

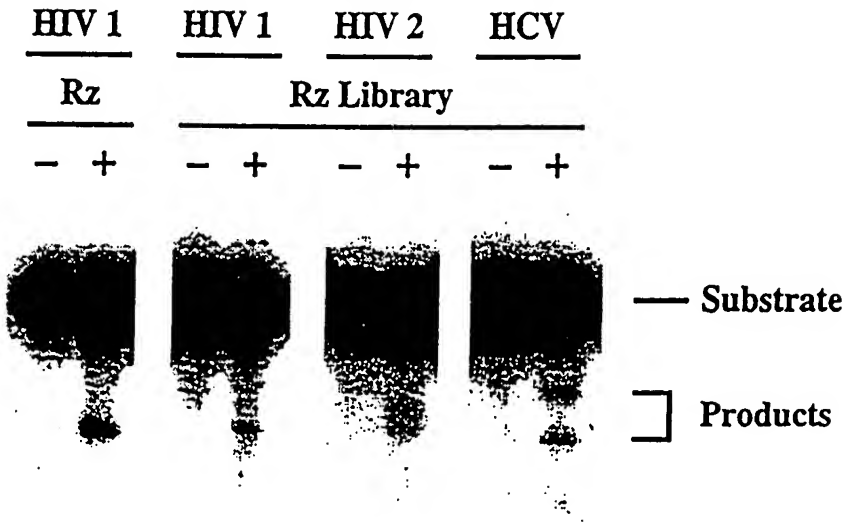


FIGURE 3

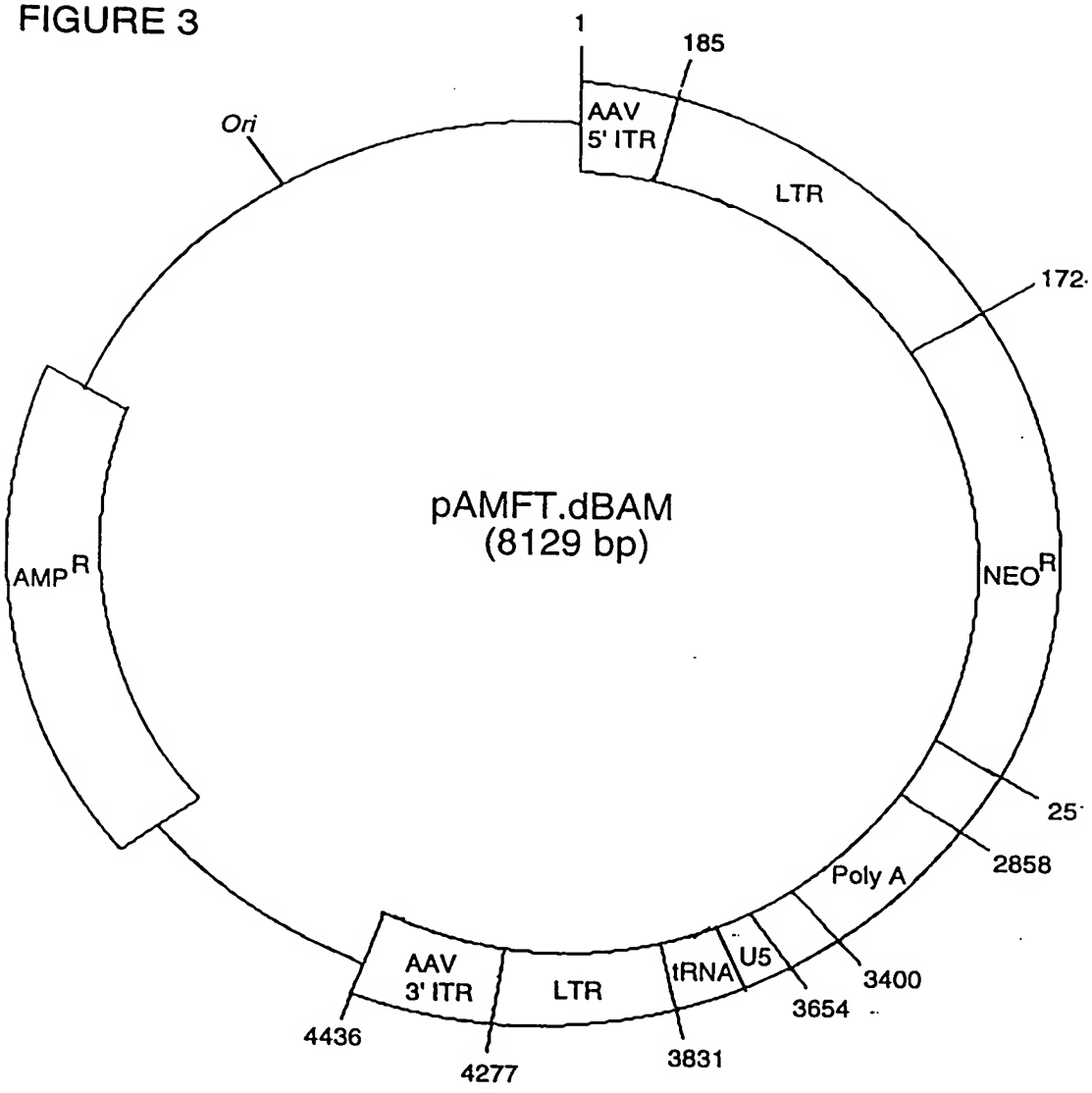

1
185
AAV
5' ITR
LTR
172
25
2858
Poly A
3400
3654
U5
tRNA
3831
LTR
4277
AAV
3' ITR
4436
AMP R
Ori
pAMFT.dBAM
(8129 bp)
NEO R

FIGURE 4 Generation of rAAV-RZ-lib provector by PCR

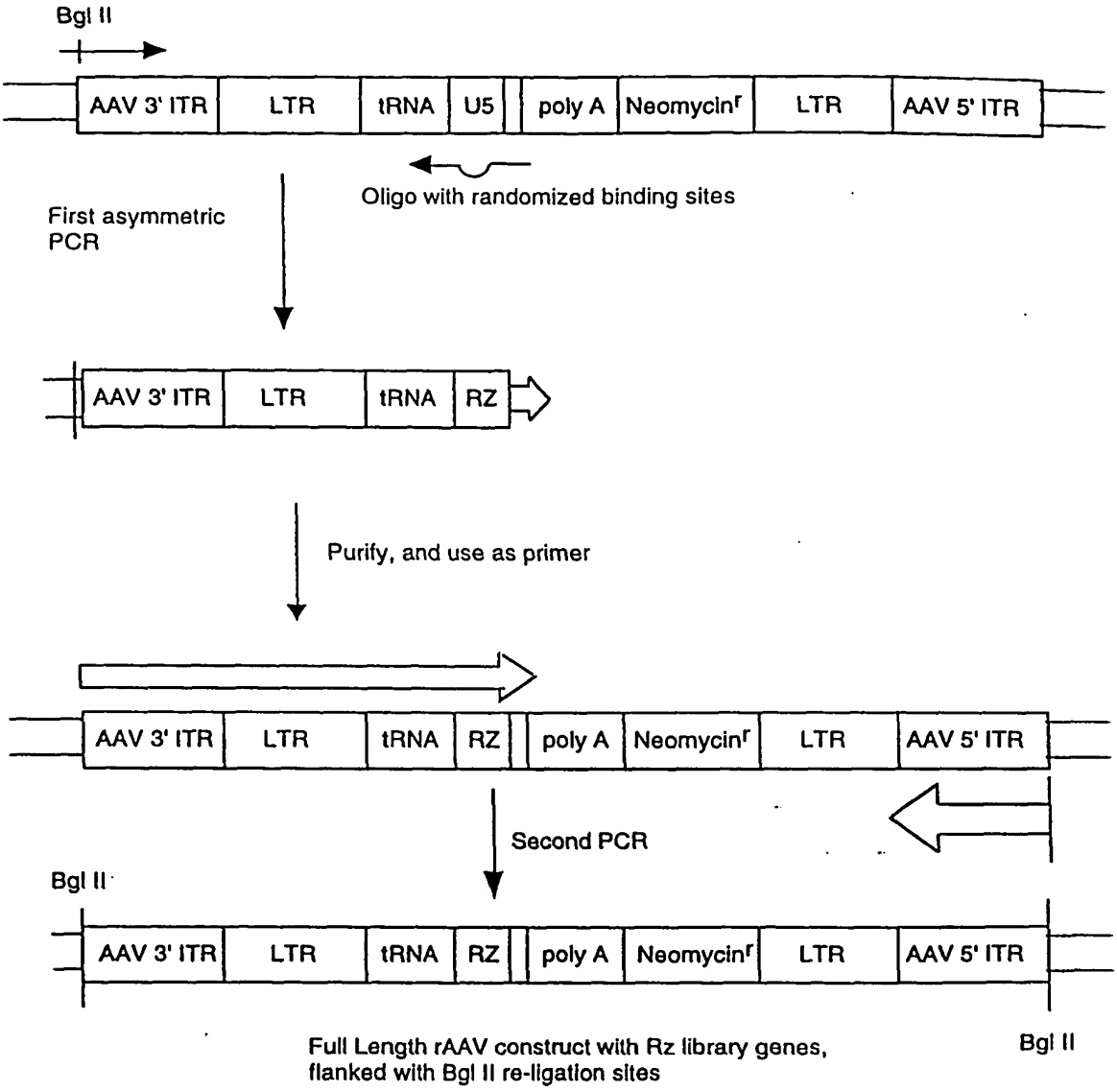


FIGURE 5

PRODUCTION SCHEME FOR ADENO-ASSOCIATED VIRAL VECTOR

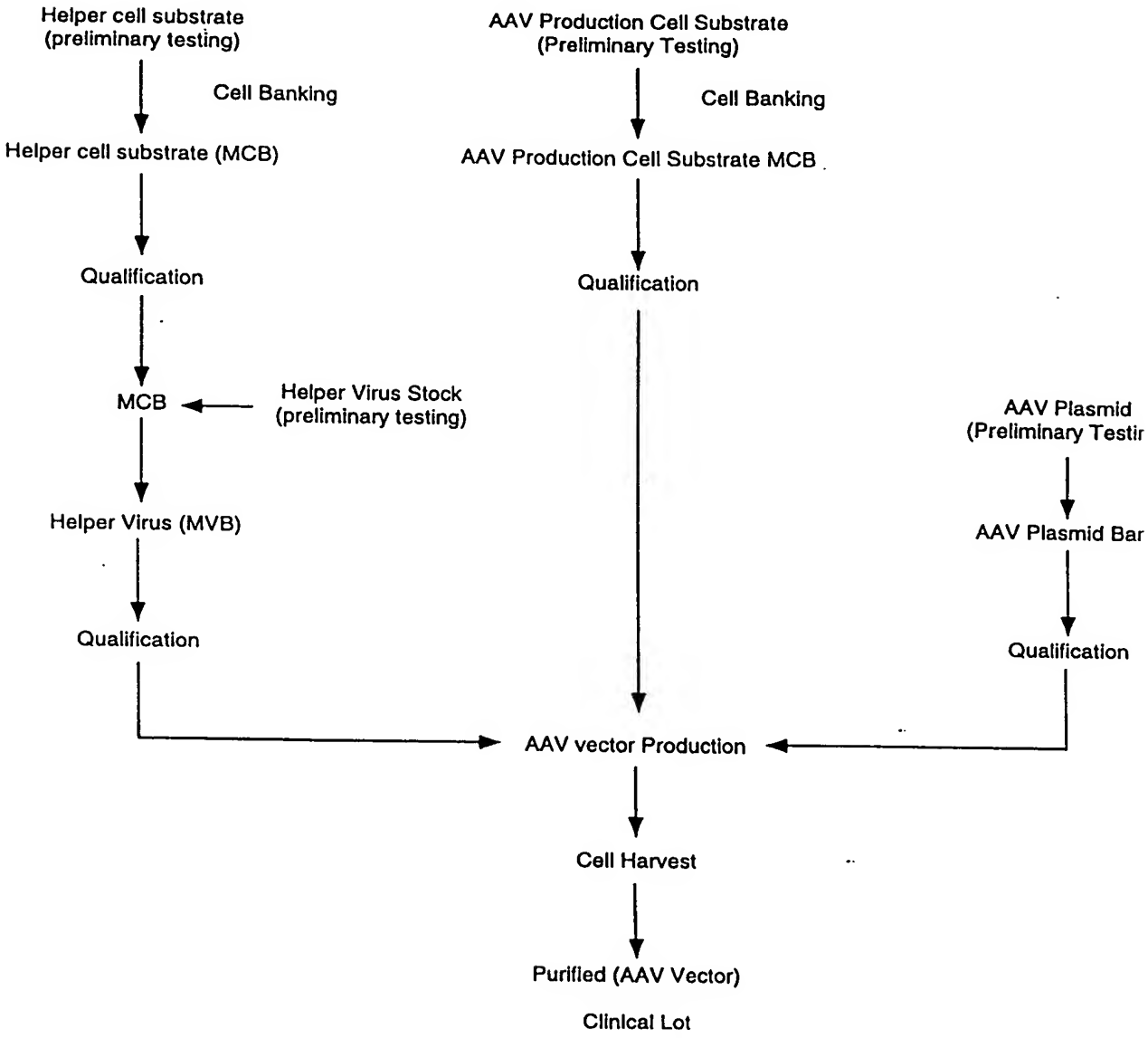


FIGURE 6 Concept of cloning genes using AAV-Rz library

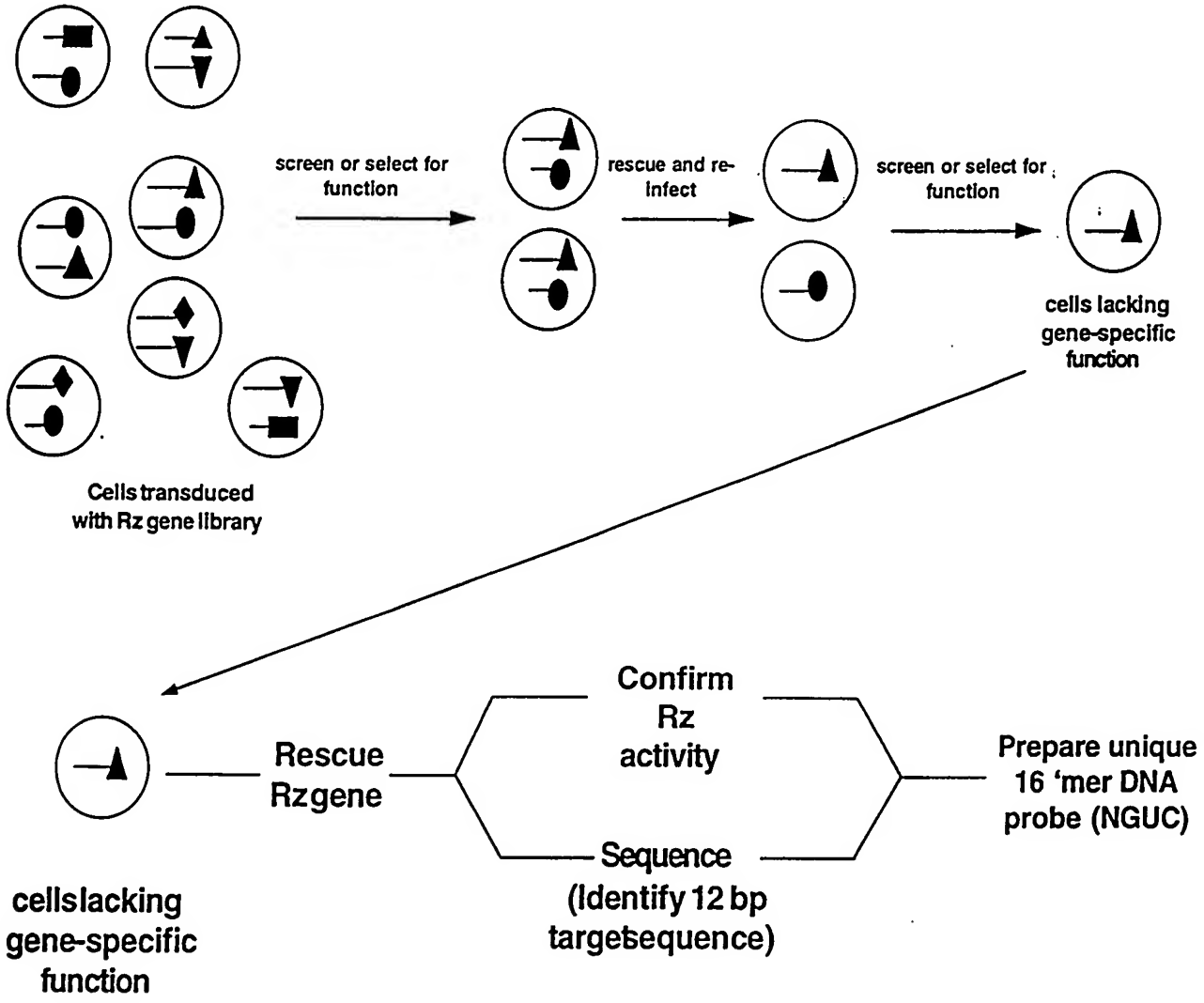
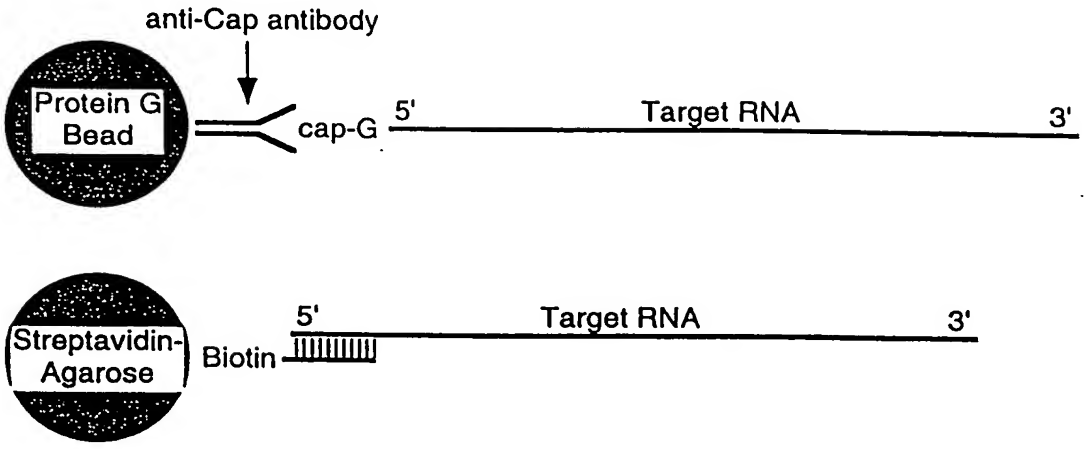


FIGURE 7 Attaching RNA target to solid support

Binding target RNA at 5' end:



Binding target RNA at 3' end:

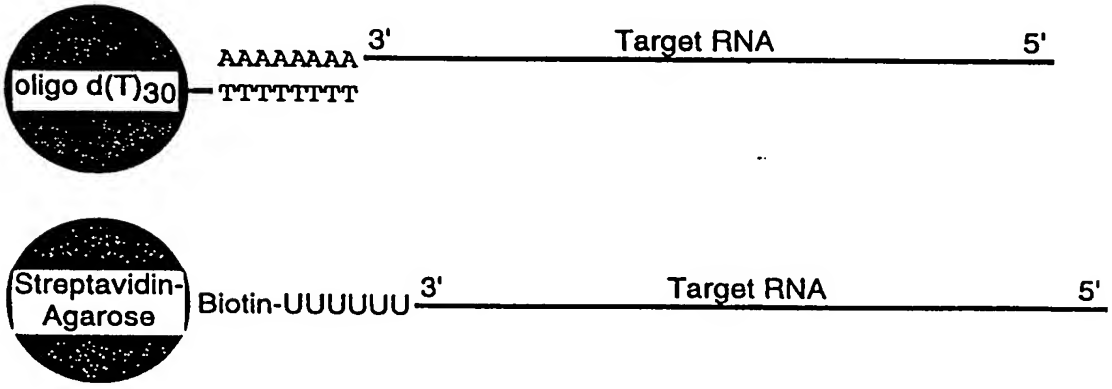
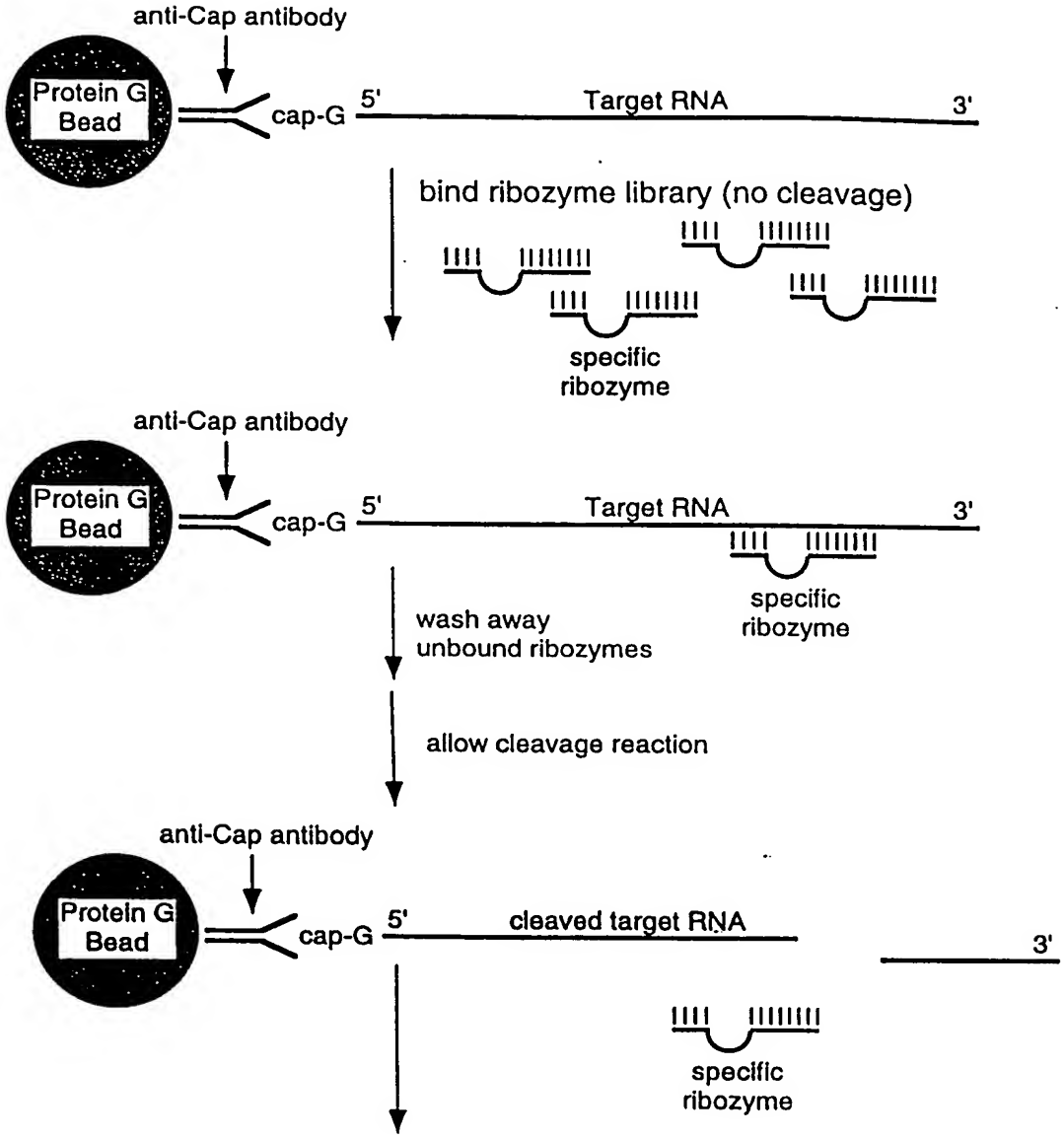


FIGURE 8 In vitro selection of optimal ribozymes



Specific ribozymes elute off the solid support

Amplify, synthesize and re-apply ribozymes to new column

Carry out selection multiple times, as necessary

FIGURE 9 AAV stable integration

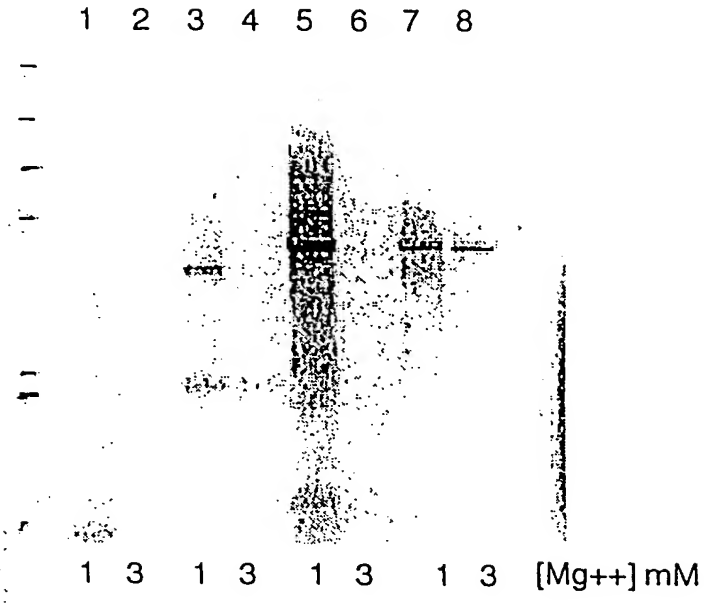
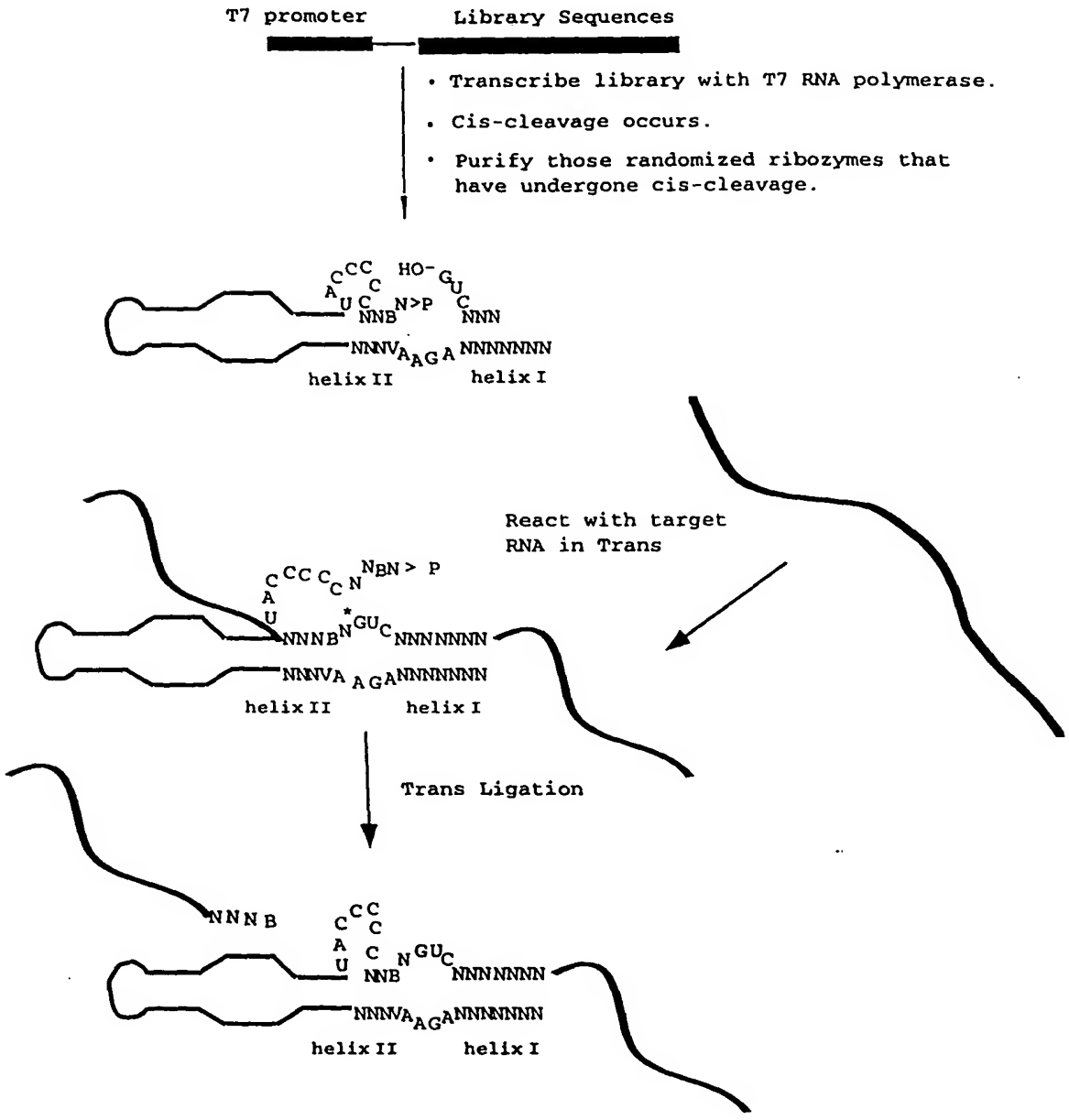


FIGURE 10 Trans Cleavage and Ligation



- Trans-ligated products are isolated and amplified by RT-PCR.
- Trans-ligated ribozymes can then be further amplified and subcloned into AAV vectors for production of a target specific ribozyme gene vector library.